PEI + ~~Ab~~ $(NH_4)_2SO_4$ ppt.

Page N

06/15/98

an to continue with purification - following the ime protocal as in wild type Tne - p.108.J

11 - 4.8mL $(.05)(6.8) = 2M\,x$ $x = 114\,\mu L$ of 2M KCl

3'exo - 4.8mL 3.8 $(.05)(4.8+x) = 2M\,x$ $x = 97.4\,\mu L$ of 2M KCl

7.0

$(40\%)(4.8+x) = 10\%\,x$

$291\,\mu L = x$ $x = 291\,\mu L$ 10% PEI

$(.40)(3.9+x) = 10\%\,x$

$143\,\mu L = x$ $x = 143\,\mu L$ of 10% PEI

Make each a final 50mM KCl slowly add ~~PEI~~ a 10% PEI sol'n to a final [] of .4%. vortex - let shake 30 minutes @ 4°C. spin in 2mL epperdafs in micro-centrifuge 20minutes @ 4°c - Save Supernatant -

60% $(NH_4)_2SO_4$ fractionation

TYI $\frac{36g\ solid}{100mL} = \frac{x}{4.8mL}$ 2.45g

3'-5 exo- $\frac{36g}{100mL} = \frac{x}{3.5mL}$ 1.26g

Vatex - let shake 30min @ 4°C
spin in SS-34 - 20,000 x g - -
Decant + Save Supernatant - Pellets

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Mary Longo	6/20/95	Recorded by	06/14/95

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140 Book No. TITLE 2mL DSO Heparin.

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Bump Heparin with .5M NaOH - Wash w/ H2O extensive
Equilibrate w/ Buffer A

Buffer A - Heparin -

25mM Tris pH 7.4
10% glycerol
5mM Bme
.1mM PMSF
.1mM EDTA
10mM KCl
conductivity - 1.2mS

Buffer B - Heparin

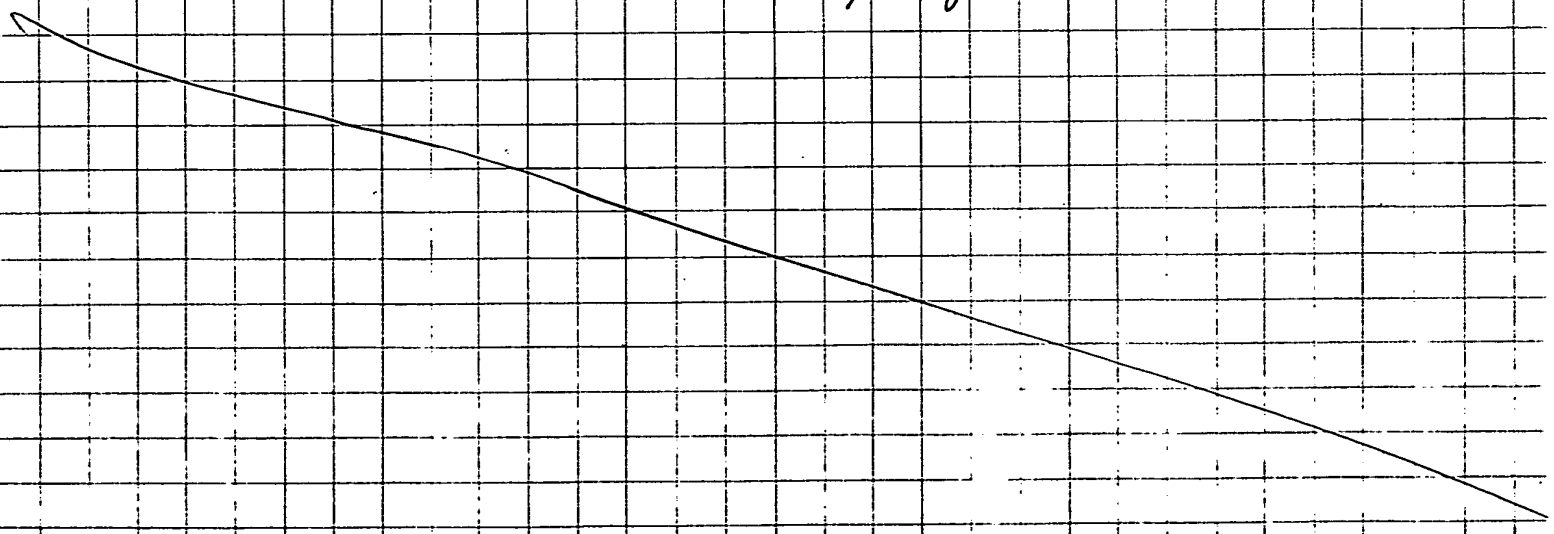
25mM Tris pH 7.4
10% glycerol
5mM Bme
.1mM PMSF
.1mM EDTA
1.5M KCl

T1-1 - Dissolve A.S. Pellet in 10mL of Buffer A — 4.5mS - conduc

Add 30mL additional of Buffer A — 2.1mS - condu

Load 9 35mL on 2mL DSO Heparin @ .25mL/min
collect flow through material - wash to base line -

Gradient Program - 0 - 100% B @ .5mL/min - 20mL linear g
wash 100% B - 10mL - @ .5mL/min
collect 500µL fractions -



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Witness d & Understood by me, Dat 6/20/95
Inv nted by Rec rd d by Dat 6/15/95

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6/15/95

Mix Rxn

Stock	For 20mL
5M TAPS	1mL
50mM $MgCl_2$	800µL
2M KCl	500µL
.1M DTT	200µL
10mM dNTPs	400µL
Fct. Salmon Testes	5mL (12.1)
	20mL s

- 1.1mL dCTP/rxn

Aliquot 500µL / tube Store in -20°C freezer - yellow tubes -

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Mary Torgo	6/20/95	Recorded by E Hagen	6/16/95

[redacted]

Project No._____
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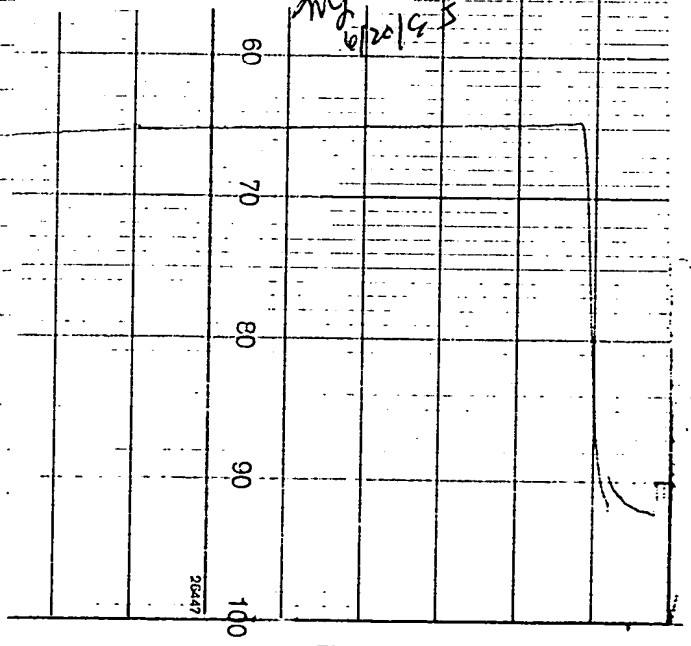
06/15

SAM	CPM1	
1	115552.0052	
2	53328.0054	
3	9146.0056	
4	4556.0058	
5	1260.0059	
6	3744.0060	
7	1028.0061	
8	574.0062	
9	536.0063	
10	346.0064	
11	730.0065	
12	438.0066	
13	348.0067	
14	21268.00	Load
15	668.00	44
16	372.00	46
17	866.00	48
18	74836.0050	
19	146.00	

EX FX-1

Pool 49-55 dialyze O/N in Q650 Buffer A

MY 6/20/95



Pharmacia LKB Biotechnology

24 µl Rxn
1 µl pack
sample -
incubate @
for 8' - qui
w/ 10 µl of 5
EDTA - SP
20 µl on E
wash
5' 1x 10% TC
3' 3x 5% T
2 + E to
dry + cou
ecoNoflour

Pool - 49
dialyze O/
in again
Q650 Buf
see p. 144

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Witnessed & Understood by m ,	Date	Invented by E Thym	Date
Mary Fong	6/20/95	Recorded by	06/16/95

Heparin 3-5 exo mutant

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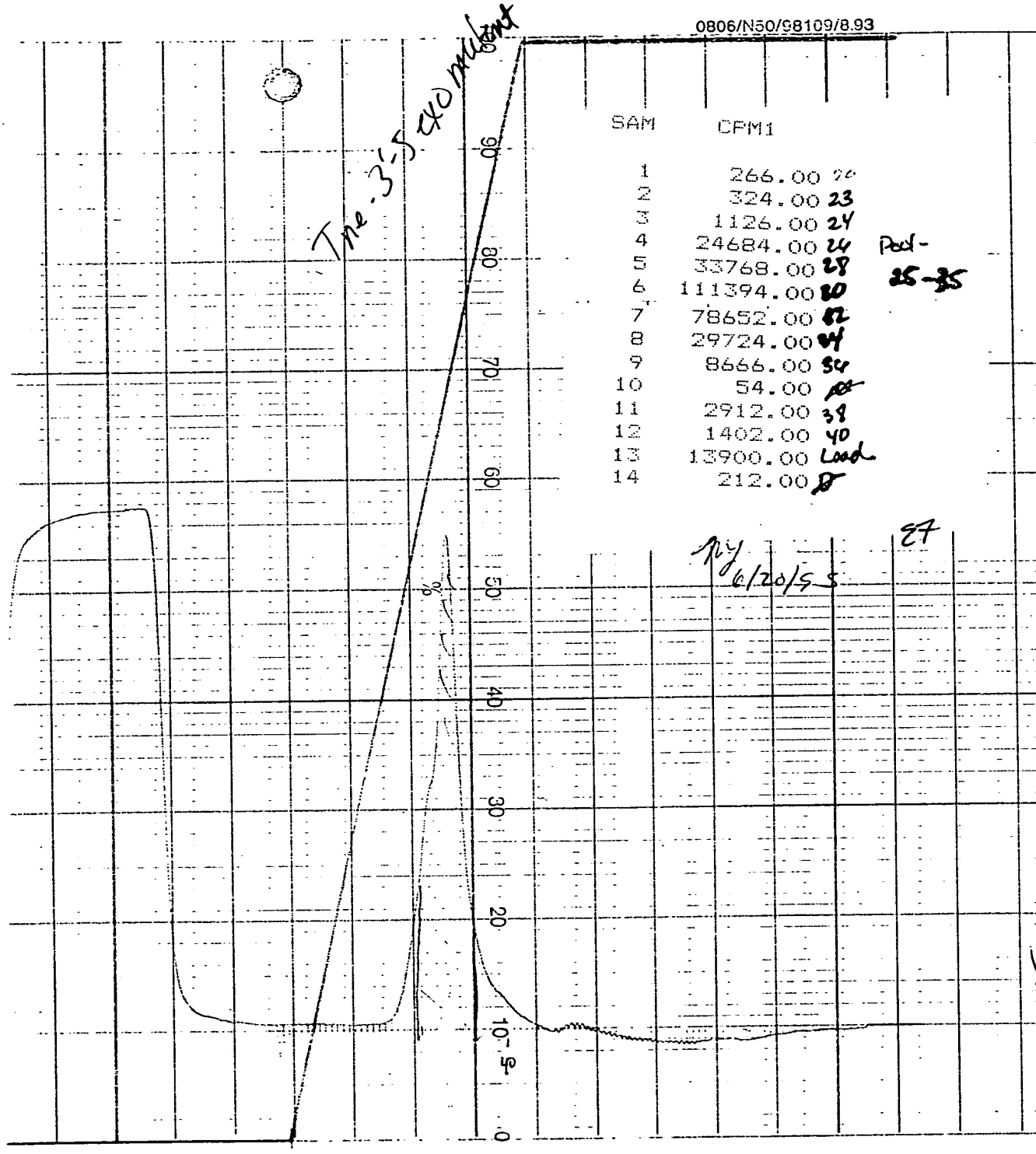

6/20/95

24 µl mix
1 µl fraction
sample -
incubate @
74° c 8 min -
quench w/
10 µl q. 5M
EDTA -
spot 20 µl
on GF/C
wash -
1x 10% TCA
1% Pi
3x 5% TCA
2x EtOH
dry +
count -

Pool - 25-35
dialyze 4 hrs
in Q 150
Buffer A =>
see p. 144

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Code No. 18-1001-44

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	6/20/95	Recorded by	06/16/95